taining capillary function since a minimal rate of edema formation was observed which did not vary appreciably with flow. (3) Hind limbs perfused with 0.88 to 2.60 vol. % oxygen exhibited augmented edema formation which increased with increased flow. The critical hypoxemia level affecting capillary permeability is believed to lie between 2.6 and 5.5 vol. % oxygen content for the experimental conditions described.

Received October 15, 1951. P.S.E.B.M., 1951, v78.

Utilization of Ammonium Nitrogen for Growth and Toxin Production by a Strain of *Corynebacterium diphtheriae*. (19143)

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In general it has been believed that *Corynebacterium diphtheriae* utilized only nitrogen from organic materials. However, it has been shown by Drew and Mueller(1) that the Toronto strain of Park-Williams no. 8 readily utilizes ammonium nitrogen in addition to that from organic substances. On chemically defined medium it was found that in the presence of optimal concentrations of amino acids growth was poor without added ammonium sulfate or ammonium chloride. The fact that glutamic acid was an essential amino acid for the test strain and that the addition of ammonium salt resulted in greater growth suggested that perhaps ammonia was being utilized to convert glutamic acid to glutamine. Hac, Snell and Williams(2) and McIlwain(3) have demonstrated that certain microorganisms readily perform the conversion of glutamic acid to glutamine when ammonia is available. The possible role of ammonia in the conversion of glutamic acid to glutamine by the Toronto strain has therefore been investigated.

Quantitative studies involving the utilization of ammonium nitrogen for the growth of the Toronto strain of Park-Williams no. 8 are to be reported. It will be shown that glutamine in high concentrations can substitute for glutamic acid and ammonium sulfate for the growth of the test strain. Glutamine plus ammonium ion has a greater additive effect than glutamine alone, indicating that ammonia is used in some other way, or in addition to glutamine synthesis.

Material and methods. A chemically defined medium known to support excellent growth of the Toronto strain and the production of high titer toxin was employed. The composition and preparation of the medium as well as the methods for cultivation are the same as previously described by Drew and Mueller(1). Total bacterial growth was estimated on the washed cells according to the Pregl micro-Kjeldahl method as adapted by Mueller(4) and expressed as "mg of bacterial nitrogen".

Experimental. Several experiments were carried out in which the growth curves produced by glutamic acid (sodium glutamate) were compared with and without ammonium sulfate added to the basal medium, and glutamine was tested as a substitute for sodium glutamate and ammonium salt. It was found that the basal medium to which sodium glutamate was added did not support good growth of the test strain and that sodium glutamate metabolism was accompanied by an accumulation of alkali (decarboxylation with the formation of (Na_2CO_3 and $NaHCO_3$)). Optimum growth took place on medium containing sodium glutamate plus ammonium sulfate, and it was observed that the presence of the

1. Drew, R. M., and Mueller, J. H., *J. Bact.*, 1951, v62, 549.

2. Hac, L. R., Snell, E. E., and Williams, R. J., *J. Biol. Chem.*, 1945, v159, 273.

3. McIlwain, H., *J. General Microbiol.*, 1948, v2, 186.

4. Mueller, J. H., *J. Bact.*, 1935, v29, 383.

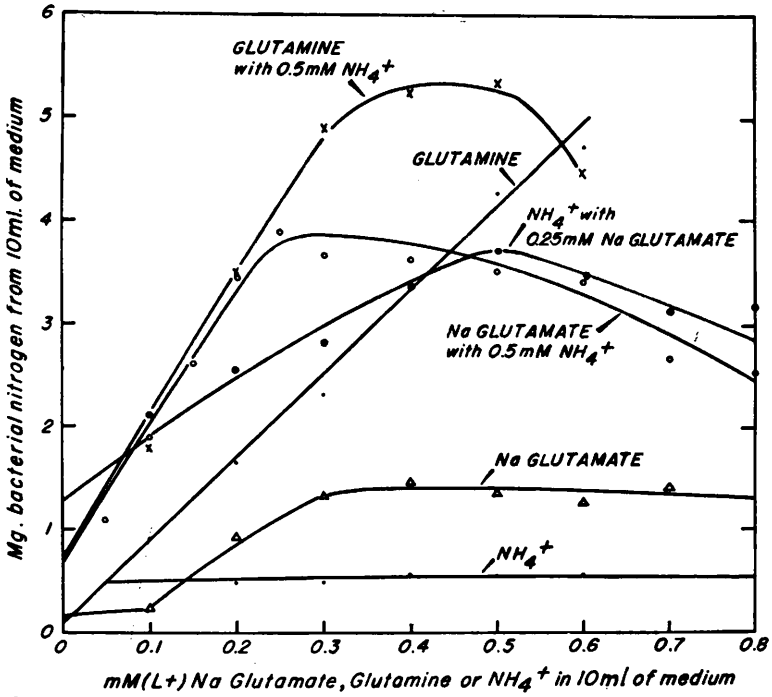

FIG. 1. Growth curves resulting from the utilization of sodium glutamate, glutamine and ammonium ion.

ammonium salt prevented the accumulation of alkali resulting from sodium glutamate metabolism. If one postulates the utilization of ammonium nitrogen and the simultaneous production of acid (H_2SO_4) as a result of ammonium sulfate metabolism, the observed neutralizing effect of the ammonium salt is readily understood. This postulation is further supported by the finding that ammonium sulfate metabolism results in the production of acid. Basal medium, to which only ammonium sulfate had been added, did not support good growth of the test organism. On this medium a small amount of growth occurred during the first 24 to 48 hours of incubation. However, cessation of growth was observed following this initial period and was attributed to the acid environment (pH 5.5-6.0). By careful selection of the amount of ammonium sulfate and sodium glutamate used, the environmental pH can be readily maintained within the range (pH 7.0-7.4) best suited to the growth of the organism. Maximum growth was obtained on basal medium containing 0.25 mM of sodium glutamate and 0.5 mM of NH_4^+.

Glutamine could be substituted in the medium for ammonium salt and sodium glutamate. Growth increased in proportion to the concentration of glutamine, and at high levels (0.5 and 0.6 mM per 10 ml medium) glutamine was more efficient than ammonium sulfate and sodium glutamate. Glutamine metabolism was not accompanied by any real accumulation of acid or alkali; the pH of the medium remained within the range of neutrality during the entire growth period. Glutamine plus ammonium ion had the greatest growth promoting effect. Fig. 1 shows the growth response to the various metabolites tested.

The ammonium salt of glutamic acid was then tested for its ability to support growth and toxin production. Ammonium glutamate was prepared by titrating a solution of l-glutamic acid with ammonium hydroxide to pH 7.0-7.2. The amount of ammonium hydroxide used was calculated to be sufficient to neutralize one carboxyl group of the glutamic acid. Growth and toxin production were determined experimentally on basal medium with added ammonium glutamate, and it was

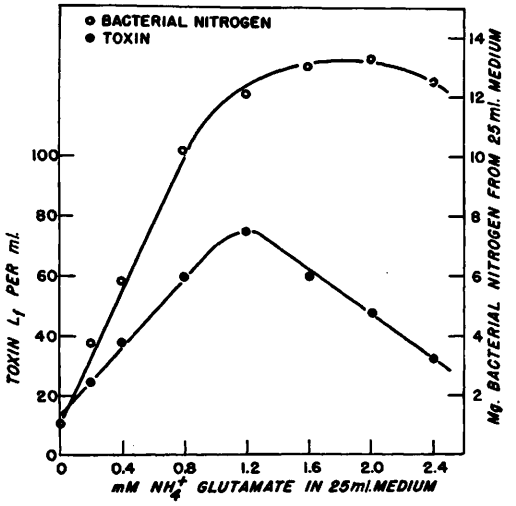

FIG. 2. Growth and toxin production on basal medium with added ammonium glutamate.

observed that the test organism grew exceptionally well on media containing 0.8 to 2.4 mM of ammonium glutamate, as evidenced by the rapidity of pellicle formation. Fig. 2 indicates that ammonium glutamate was readily utilized by the Toronto strain for growth and toxin production. Toxin production reached a maximum on medium containing 1.2 mM, but fell off sharply with increasing amounts of ammonium glutamate; the decrease in toxin production was probably due to inhibitory amounts of iron since the particular lot of glutamic acid used contained iron as impurity. The extreme sensitivity of the toxin-producing mechanism of the diphtheria bacillus to traces of iron is well recognized. In repeated experiments on iron-controlled medium, ammonium glutamate has been found to be equally as good as the combination of ammonium sulfate and sodium glutamate for growth and toxin production. Glutamine has consistently proved to be less efficient for toxin production on a low iron medium.

Discussion. The experimental results do not warrant the conclusion that glutamine is the metabolite required, and that in the absence of glutamine, ammonia and glutamic acid are essential for the synthesis of glutamine. The fact that high concentrations of glutamine are required for maximum growth makes it seem more probable that glutamine is broken down by the organism to ammonia and glutamic acid, which, of themselves, are readily utilized in a favorable pH environment. This conclusion is supported by the evidence that ammonium glutamate proved to be an efficient substitute for ammonium sulfate and sodium glutamate, or glutamine. The apparent synergism between ammonium salt and sodium glutamate may be simply a function of pH since the addition of ammonium sulfate prevents the accumulation of alkali resulting from sodium glutamate metabolism. Likewise, the acidity resulting from ammonium sulfate metabolism can be prevented by the addition of sodium glutamate.

Summary. The utilization of inorganic nitrogen in addition to organic nitrogen by the Toronto Strain of Park-Williams no. 8 *Corynebacterium diphtheriae* has been investigated. Ammonium nitrogen in the form of a simple ammonium salt (ammonium sulfate) is shown to be utilized by the organism. For optimum growth and toxin production on chemically defined medium, suitable concentrations of sodium glutamate and ammonium sulfate, or ammonium glutamate are required.

Received October 17, 1951. P.S.E.B.M., 1951, v78.

Effect of Sanguinin, an Antibacterial Agent from Blood,* on Streptococcal Infection in Mice. (19144)

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In our earlier studies(1) the antibacterial activities of tissue extracts of several species of ticks were demonstrated. It was found that

* Patent pend.

1. Anigstein, L., Whitney, D. M., and Micks, D. W., *Tex. Rep. Biol. and Med.*, 1950, v8, 86.